Introgression between *Primula elatior* (L.) Hill. and *Primula officinalis* L.*

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INTRODUCTION

The taxonomy of species from the genus *Primula* in the section *Vernales* has always been difficult. Many early authors, Darwin among others, pursued a prolonged scientific investigation of the *Vernales*. According to Wright-Smith and Fletcher (1947), several species consist of five or eight forms differing only slightly in morphology and growing in fairly distinct geographical areas, and therefore classed as subspecies. Valentine (1953) stated that "many of these subspecies are somewhat inaccessible and only a few have been investigated at all closely, so that their status is uncertain".

Although some artificial hybridization between members of the genus has been reported to date, few cases of natural hybridization have been recognized.

This paper deals with a case of introgressive hybridization between two species from the section *Vernales* of the genus *Primula*, namely *Primula elatior* (L.) Hill. and *Primula officinalis* L.

Both species have a wide geographical distribution and are sympatric over the greater part of their respective ranges. The numbers of chromosomes are the same in each case $2n=22$. They are perennials and hemi-cryptophytes. The leaves grow in rosettes, the scape has at the top an umbellate inflorescence, and the flowers are characterised by their heterostyly. Both species differ in numerous vegetative and generative characters.

In Poland *P. elatior* grows principally in the Tatra and Carpathian mountains and to some extent in lower regions while *P. officinalis* is dispersed mostly in the lowlands and does not grow in the mountains (Pawłowski 1963). Their distribution in Poland is to a considerable degree overlapping; therefore it might be supposed that introgressive hybridization occurs.

The Tatra National Park was chosen as a locality of the population

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of *P. elatior*, and the Wielkopolski National Park for *P. officinalis*. This selection of localities was made because the population of each species was growing in the absence of the other, and so were regarded as pure populations.

In the Ojców National Park, the distribution of the two species is adjacent and overlapping. The most common there appeared to be natural backcross hybrids.

In artificial hybridization between the species studied, many difficulties were encountered, as will be described later. The results of experiments made by Valentine (1957—1961) were taken into consideration.

In the present work, the investigation of natural populations is principally dealt with.

MATERIAL

In 1955, collection was started of the first living plants, and they were grown in the Kórnik Arboretum. The cultures included plants transplanted from natural habitats of the species studied.

In the spring of 1961, at flowering time, the first detailed observation of the natural populations was made in order to select localities and to label the individuals. In the fall of 1961, a total of 539 plants were examined, including the following:

<i>P. elatior</i>	94 individuals
<i>P. officinalis</i>	101 individuals
Population 200	46 individuals *
Population 500	49 individuals
Population 300	82 individuals
Population 400	78 individuals
Population 600	89 individuals

In the spring of 1962, 304 of the above plants were examined with the following number of individuals from each population:

<i>P. elatior</i>	45 individuals
<i>P. officinalis</i>	42 individuals
Population 200	43 individuals
Population 500	42 individuals
Population 300	32 individuals
Population 400	55 individuals
Population 600	45 individuals

* The samples of each population were labelled from 1—99 for *P. elatior*, 100—199 for *P. officinalis*, and 200, 300, 400 etc. for the populations of the Ojców Nat. P-k. The names of the populations were shortened to 200, 500 and so on.

Fig. 1. Map of the areas where the studied natural populations grew

I. Description of parental species

P. elatior (L.) Hill.

a) Source of material. The locality was situated in the Tatra National Park at an altitude of 700 metres above sea level, south of Zakopane on a sloping area of a mountain covered with spruce and fir, with some intermixture of beech and maple, adjacent to a meadow where the stream Biały intersects the northern boundary of the National Park. In this area, no other species of *Primula* occurs, so that the possibility of using hybrid material was excluded (Fig. 1).

b) Description of plants. (Fig. 2). Height from 6—24 cm; one to three scapes with 2—12 flowers in the largest inflorescence. Ten to eleven leaves, forming a rosette, developing first in the outer part, the inner part growing latest. The leaves developed between the outer and inner part are largest at the time of flowering, so that the length of the largest leaf at flowering time is 2.5—15.0 cm, and in the fall

Phot. Z. Zwolińska

Fig. 2. A plant from the natural population of *P. elatior*

6—20 cm, its width 1.5—5.5 cm and 3.0—8.0 cm respectively. Leaves are tomentose and distinctly cordate early in development. Pedicel 0.4—2.2 cm. Flowers open with sepals slightly recurved, corolla diameter 0.9—2.4 cm. Colour of petals — sulphur and primrose yellow (No. 1 and 601 according to the Horticultural Colour Chart); colour of throat — canary, lemon yellow and aureolin (No. 2, 4, 5). The pattern of the throat is characteristically ring-shaped. The calyx is pear-shaped, 1.0—1.6 cm long at the time of flowering and 0.7—1.7 cm in the fall; width 0.3—0.6 cm and 0.4—0.7 cm respectively. Capsule length 1.0—1.9 cm, and width 0.4—0.6 cm. From 1—13 capsules are developed in the fall (Plate I, II).

P. officinalis L.

a) Source of material. The plants grow in the Wielkopolski National Park situated about 15—20 km south-west of Poznań. The spot is on a small hill on the southern shore of the Góreckie Lake, south east of the road from the village Jeziory to Puszczykowo. On the hill grow oaks and pines (Fig. 1).

b) Description of plants. Height 18—42 cm. One to seven scapes with 3—14 flowers in the largest inflorescence. Leaves, developing as in *P. elatior* in the rosette, but mostly obovate and puberulent. Length of the largest leaf at flowering time 5.7—30.0 cm and in the fall 18.0—36.0 cm; width 3.5—9.0 cm, respectively. The pedicel is from 12 to 38 cm long. Flowers have corolla diameter 0.9—1.7 cm. Petals are lemon and canary yellow (No. 4,2) and the colour of the throat ranges from aureolin through buttercup and saphron yellow to nasturtium orange (No. 3, 4, 5, 7, 14). The pattern of the throat consists of 5 spots. Calyx is campanulate and 1.3—2.1 cm long in the spring, 1.0—2.0 cm in the fall; width 0.6—1.1 cm respectively (Pl. I, II). Capsule length 0.4—1.3 cm, and width 0.3—0.9 cm. From 5—25 capsules were developed in the fall (Fig. 3).

Phot. K. Jakusz

Fig. 3. A plant from the natural population of *P. officinalis*, 1/4 nat. size

In addition to the traits mentioned above, the two species differ in many other respects, such as for instance: time of flowering and ecological requirements.

II. Description of the population from the Ojców National Park.

On the basis of observations of comparative external morphology, there were found in the Ojców National Park, situated about 30 km north-west of Kraków, at an altitude of 350 m above sea level, some areas in which were growing groups of backcrossed individuals in company with their putative parents. The Ojców National Park is on a rocky mountainous area of Juratic origin. It is a region of overlap of the habitats of the species studied. In this area, 5 localities were selected for 5 different populations.

Localities

Population 200. This is situated on the north-west slope of the mediaeval castle hill in the village of Pieskowa Skała, which is 6 km north of Ojców. The slope is on the north-east side of the gorge, where runs a road connecting the main Kraków—Olkusz highway with the village of Wielmoże. About 112 m from the main road the locality can be found, on the slope. It is a shaded hornbeam wood with ash-trees and some scattered maples. The situation is rather moist.

Population 500. This locality lies on the opposite side of the gorge. It is situated on the southern-west slope of the hill, some 55 m. from the main road and 20 m up the hill. This place is slightly shaded and dry, covered by rare specimens of hornbeam, and principally ash-trees. It is separated from the one referred to above by the road, and from the locality of the next population (400) by a belt of heaps of rocky stones. At midday it is a sunny spot.

Population 400. The locality is to be found on the same hill, as the last-mentioned, but some 40 m east of it. The place extends from the base of the hill $\frac{2}{3}$ up the slope, and in its central part is rather flat; there are, in addition to hornbeams and ash-trees, some birches. It has a rather dry soil.

Population 300. This locality was chosen lying on the southern side of the hill in Pieskowa Skała. It is adjacent to the wall surrounding the castle court-yard from the side of the main road from Kraków to Olkusz. This is a rocky place, and rather dark. Found there are old hazel tickets and a few hornbeams, maples and cornelian-cherries.

Population 600. The locality was chosen at a distance of some 2 km from Pieskowa Skała, in the direction of Ojców. The area belongs to the village of Młyny, which is situated to the north-east of the main

road to Kraków. There is a large valley, connecting the village of Młyny with Wielmoże. The locality is to be found on the slope of a rocky hill, on the north-west side of this valley, at a distance of some 70 m east from the main road. It is a rocky place, covered with hazel shrubs and small junipers. The area is sunny and dry.

Description of plants (Plates I and II).

Population 200 (Fig. 4). The plants of this population are rather uniform, do not differ significantly one from another, and are as to most of their characters similar to *P. elatior*. The colour and the pattern of the corolla as well as the shape of the calyx are almost the same; at the first sight, therefore, it appears to be a population of *P. elatior*. In dimensional characteristics they sometimes present, however, striking differences. The leaves are larger and this is particularly apparent at the time of flowering: length 5.7—3.0 cm, and width 1.5—6.5 cm. The indumentum is more intensive. The calyx and the capsule are smaller, 0.7—1.7 cm and 0.6—1.1 cm, respectively. Height 10—30 cm. One to five scapes per rosette and more; 4—18 flowers in the largest inflorescence. The corolla is smaller, 1.1—2.1 cm. There are more capsules developed 1—15. The size of the seeds does not differ significantly.

Phot. J. Poszwińska

Fig. 4. A plant from the natural population 200. 1/2 nat. size

Population 500 (Fig. 5). The individuals of this population are also rather uniform, but they resemble the other species — *P. officinalis*. They do not differ significantly at first sight from that species, and therefore are usually treated as its representatives. They differ slightly as to colour of corolla, but not in throat pattern and calyx shape. Nevertheless, the dimensional characters present in some instances

Phot. A. Jasiewicz

Fig. 5. A plant from the natural population 500

marked differences. By comparison with *P. officinalis* the leaves are smaller. Their length is 5.0—22.5 cm, and width 0.5—4.5 cm. The plants are more tomentose. Height is less, 18.0—38.0 cm, and there are developed less capsules, 1—17 and less seeds in the capsule.

The populations 300, 400 and 600 are different from those mentioned above as to general characters. They are much more multiformed. Their samples show a relatively high proportion of values intermediate in characteristics in comparison with the traits of the two species studied. There are to be found among them phenotypes almost similar to the typical for the species, as well as different types of segregates with several combinations of characters.

Population 300. In this population one meets individuals very similar to *P. elatior*, as well as others resembling *P. officinalis* and some

of the intermediate type in the nearest vicinity. Though in most characters similar to *P. officinalis*, the dimensional characteristics are intermediate or tend to resemble *P. elatior*. From the 40 specimens examined 28 have lemon and canary yellow corolla, only one being primrose, two buttercup yellow, and 9 aureolin. The colour of the throat is also darker. Specimens totalling 38 have shades from buttercup and saphron yellow to nasturtium orange, and only two in the canary yellow tints. Most are marked with *P. officinalis* spots, and the shape of the calyx is campanulate. Height 10—36 cm. Leaves rather small 5.7—25.0 cm in spring, and 12—28 cm in the fall, but indumentum like *P. officinalis*. Number of scapes 1—3 and 2—10 flowers on inflorescence, much as in *P. elatior*. The corolla diameter smaller 1.0—1.8 cm. The capsules are short, but rather large 0.5—1.0 cm. and 0.4—0.7 cm respectively. From 1—13 capsules developed, almost as in *P. elatior*. The size of the seeds is generally intermediate between the species studied, but some are of a different size.

Population 400. Of all population studied, this one contains most individuals of mixed characters from the two species. Among the plants resembling the two species, there are some the characters of which are intermediate between the two putative parents, and they are more numerous than in populations 300 and 600. It is evident from specimens No. 410 and 411 how differently the qualitative and quantitative characters of the two species are mixed. These two plants are apparent F_1 hybrids.

In general, in this population the leaves are not very long — from 5.0—22.5 cm in spring, 8—26 cm in the fall, and 1.5—7.5 cm wide respectively. Height 16—40 cm. One to four scapes; the number of flowers from 2—21; most varied of the populations studied. Capsule rather short and large. The numbers of capsules developed are intermediate or small, as in *P. elatior*. The numbers of seeds are varied. Some plants have seeds of varying size.

Population 600. In this population, the prevailing types are similar to *P. elatior*, and there are less samples resembling *P. officinalis*. Groups of plants similar to *P. elatior* occur growing together. They may be backcross hybrids which are very similar to the putative parents from a morphological point of view, so that in the field it is very difficult to make out the difference between them. As applied to such cases, Clifford (1958) in his paper on "Introgression between Primrose and Cowslip" says "they could easily be overlooked in the field".

In general, in this populations, there are clear-cut differences between each phenotype which is similar to the pure species. Nevertheless, there are some very rare types of intermediate characters, for instance No. 687.

The leaves of this population are mostly smaller: 1.0—12.5 cm long

at full bloom and 1.0—20.0 cm in the fall with width of 1.0—4.0 cm, and 0.5—6.0 cm respectively. Some of them are even smaller than those of *P. elatior*. Calyx length 1.0—1.9 cm. In 83 percent of examples, its shape is pearshaped, like the *P. elatior* type. The plants are generally small. Height 8—22 cm. From one to four scapes 2—17 cm in width. Number of capsules small: 1—13, and they are not very sound, over 50% having only 10 seeds. Seeds do not differ significantly one from another as to size.

METHODS

Crossing of parental species

Plants collected from the natural habitats in Poland, from the localities of the Tatra, and Wielkopolski National Parks were transplanted to garden beds and the cold greenhouse of the Institute of Dendrology and Arboretum in Kórnik. Emasculation was effected two days before pollination only with short-styled "thrum" flowers, used as female parents. In the long-styled plants, the emasculation was found unnecessary, since the stamens in the "pin" flowers develop later than the pistil (Valentine 1947). The plants were screened with cages of cheese cloth in the greenhouse. The emasculation of the shortstyled flowers was effected by removing the anthers from the bud with forceps. To isolate the flowers growing in the garden beds, paper bags were used. The plants were pollinated by hand pollination. Mostly long-styled flowers were pollinated by the short-styled one (legitimate) and reciprocally, and only a few long styled flowers were pollinated by long-styled pollen (illegitimate). When the seeds reached maturity they were gathered, counted, weighed and measured. After that they were sown.

Plate I

Figs. 1—7. Corollas and calyces in the samples of natural populations from Tatra, Wielkopolski and Ojców National Parks

1 — *P. elatior*; 2 — *P. officinalis*; 3 — population 200; 4 — population 500; 5 — population 300; 6 — population 400; 7 — population 600. The sequences of calyces correspond to the sequences of corollas

Plate II

Figs. 1—4. Capsules dissected in about the middle of the growth period

1 — samples from the population of *P. elatior* (Letters correspond to the No. of specimens. c — 10, b — 49, c — 62, d — 2); 2 — samples from the population of *P. officinalis*. (A — 122, B — 163, C — 145, D — 155); 3 — samples of the population 400 (M — 409, N — 410, O — 408, P — 483); 4 — samples of the population 400. (R — 411, S — 403, T — 481, U — 426)

Phot. K. Jakusz

Plate I

Analysis of the natural populations

From the populations described above, 46 to 101 individuals were marked. Each plant received a label, the greater part of which had to be dug into the soil, further three twigs were placed round the plant so that it would not be overlooked, even when covered with snow. The place where each sample was growing was described in detail and was also sketched on to a working map.

The sample individuals from the populations were examined in several seasons, mostly in spring and autumn. In the spring of 1961 the first observation was made. In autumn, herbarium material comprising the largest leaves and scapes with the dried mature seeds was gathered. On these materials were made the measurements described below. At the time of flowering some corolla specimens were collected. The corollas were removed from the flower tubes and the flower discs were fixed with sellotape (Valentine 1959, personal hints). The calyx was cut out on one side and spread out, and then fixed with a similar sellotape on a white paper. In the spring time numerous observations, descriptions and measurements of the characters listed later were made. The first seeds were gathered in the fall of 1961.

The measurements of flower characteristics were done from 25.4—7.5.1962. About 40 days after full bloom, the semimature, mostly soft seeds were collected. The peduncle of the inflorescence containing capsules with seeds were soaked in water to prevent withering. They were transplanted in vases from the natural stands to Kórník, where they were stored in a refrigerator at $t^{\circ} + 3^{\circ}\text{C}$, to prevent prompt maturation. On this material, the measurement of 12,389 seeds was made. The seeds were dried and weighed. Before measurements were taken, the seeds were soaked in water for 7—21 days, and then were squashed with a small scalpel or needle, care being taken to have the embryo squeezed out intact. The embryos separated were fixed in a solution of lactophenol with glycerol (1:2) with methyl blue. The measurements of the endosperm and material tissue layers were made in longitudinal sections, under a binocular.

After all biometrical work has been performed, the data were analyzed by conventional methods. The range of variation of particular characters, the means, standard deviation and analysis of variance were studied. Further, a special technique for the study of introgression, used by Anderson for such a process of evolution, was applied. The method of hybrid index was employed for an efficient comparison of the natural populations and in order to show the great variety of combinations of characters. To present the possible correlation between certain quantitative and qualitative characters, the method of scatter diagram was used.

ARTIFICIAL HYBRIDIZATION

The compatibility of the parental species

There are many difficulties to the crossing in nature of both the species studied. Different isolation mechanisms prevent or retard the development of the hybrids. Among the external barriers, of importance is the mechanical isolation caused by the dimorphism of the heterostyled flowers, as Darwin (1868) stated, Bateson and Gregory (1905) tried to explain, and Crosby (1949—52) and Woodell (1960) have elucidated experimentally.

Further, the seasonal isolation is rather significant. *P. elatior* in natural conditions develops earlier in the adjacent geographical and climatic areas than does *P. officinalis*; Valentine (1952) has quoted about 1 week to 1 month for English material. The differences in time of flowering for the species of the section *Vernales* are greater than in Great Britain, according to E. de Vries (1919) in Holland and Fey (1929) in Austria, on the continent. In the present work, the extent requires from two weeks to 1 and a half months. The period of overlap, when both species are in full bloom is only about a fortnight, since *P. elatior* has a shorter duration of the time of flowering.

The possible significance of ecological influences in restricting gene-flow is considered. Oxlip and cowslip have different ecological requirements. Cowslip grows rather in central parts of Poland on dry and rather sunny meadow, while oxlip develops in the mountainous parts, in moist meadows and wooded northern slopes.

As internal barriers, there are hybrid partial inviability or weakness and partial hybrid sterility. According to Vazard (1955) there are differences in timing between the moment of pollination of the pistil and the fusion of gametes. In the *P. officinalis*, this requires 45—48 hours, and in *P. elatior* 65—72 hours. According to Dahlgren (1916) and Valentine (1955), for 40 days after fertilization the embryo is still small and enclosed in the endosperm, which is not yet hardened. After this time, the endosperm becomes hard and the seed reaches maturation on about the 80th day. As a result of experiments by Harrison (1931), Crosby and Valentine (1952), hybrids between the two species studied were produced. Woodell (1960) furthered this study by way of anatomical and embryological experiments. The seeds obtained by Valentine from the cross, where *P. elatior* was used as female parent, were normal in size and almost empty at maturity; thus no hybrids yielded F_2 . When *P. veris* was the seed parent, the seeds were mostly small, but few large seeds were found with developed embryo and endosperm, which might germinate. Valentine (1956) has obtained viable F_2 generation and backcrosses.

The germination recorded for the F_1 hybrids was 0.5%, for F_2 (*veris-elat.* $\times$ *veris-elat.*) was 61%, and in backcrosses 59%. The seeds were 73% full at maturity, while for the parent forms it was 100%, and 0% for the F_1 . Valentine (1953) showed that some of the successful *P. veris* $\times$ *P. elatior* hybrids were triploids ($2n=c.33$), and in his later paper (1963) stated that these triploids were relatively easy to produce by crossing diploid *P. veris* ($2n$) σ with tetraploid *P. elatior* ($4n$) φ , but that diploid hybrids were still very difficult to obtain.

Experimental data concerning artificial hybrids

The parent species from the Tatra and Wielkopolski National Parks were cross pollinated in the years: 1955, 6, 7 and 1959. Thus, one hundred eighty nine crosses altogether were made between *P. elatior* $\times$ *P. officinalis* and the reciprocal, which yielded 6083 seeds. The comparison of the yield obtained from the parental species and the crosses is given in Table 1. The numbers of seeds are somewhat smaller on the average than those from intraspecific crosses. The measurements of seed length and contents of endosperm gave results similar to the data of Valentine (1952) and Woodell (1960). A total of about 3995 seeds from the cross *P. elatior* as female were sown, and from the reciprocal 2088 seeds. Of these, one seedling germinated in the first year, but before developing the cotyledons withered. In the next year, one seed germinated, developed and flowered. Both seedlings originated from the reciprocal cross. Therefore, it was evident that none germinated from the cross *P. elatior* $\times$ *P. officinalis*, and from the cross *P. officinalis* $\times$ *P. elatior*, a very small amount: 0.05% of seeds, less than in Valentine's experiments. The only plant, grew in isolation from the other species, and selfpollination took place. The measurements of the plants were as follows: height 15.2 cm; leaves 10.6 cm long, 5.6 cm wide; calyx 1.5 cm; corolla colour: light canary yellow (2/2 ac. H.C.C.); the spots on

Table 1

Number of pollinated flowers, of capsules developed and the mean number of seed in a capsule in the artificial crosses:

P. elatior φ $\times$ *P. officinalis* and *P. officinalis* φ $\times$ *P. elatior*

Cross	No. of pollinated flowers	No. of capsules	Mean no. of seeds
<i>P. elatior</i> φ $\times$ <i>P. officinalis</i>	122	41	30.6
<i>P. officinalis</i> φ $\times$ <i>P. elatior</i>	601	130	28.3
<i>P. elatior</i> φ $\times$ <i>P. elatior</i>	71	23	34.6
<i>P. officinalis</i> φ $\times$ <i>P. officinalis</i>	100	24	39.2

Table 2a

Seed size of the F_1 hybrid plant from the cross
P. officinalis ♀ × *P. elatior* (content of 4 capsules)

Capsule	No. of seeds per capsule	Seed length in mm				
		2.5 - 2	2 - 1.5	1.5 - 1	1.0 - 0.5	0.5
1	2	2				many
2	2		1	1		many
3	9			5	4	
4	3	3				many

the throat were marked in saphron yellow (7/1); flowers were "thrum" shaped, which was suitable for selfpollination; number of flowers — 12.

The hybrid described above revealed characters intermediate between the individuals of the stock T21e and T21h obtained as F_1 hybrids by Valentine. There were fruits and seeds from the hybrid obtained. The seeds from 4 capsules were measured (Table 2a). In the remaining

Table 2b

Seed content of F_1 hybrid of the cross
P. officinalis ♀ × *P. elatior* (from the two other capsules)

Capsule	No. of seeds: large	Content of the large seeds			
		small	embryo and endosperm visible	endosperm present, embryo not apparent	endosperm not identified, embryo very small
5	8	many	3	0	5
6	14	many	9	1	4

two capsules the content was examined, as shown at Table 2b. The seeds were sown, but were sterile; none germinated.

The occurrence of seeds larger than normal and empty in the hybrid from the cross with *P. elatior* as female, and of seed relatively small, but more developed from the reciprocal cross are in accordance with the results of the Valentine's experiments. Although the mean size of the seeds from the reciprocal cross is smaller than those having *P. elatior* as seed parent, the weight is greater, and hence the content is larger.

In 1961, crosses were made between some plants from the Ojców National Park. Individuals of the population 200 were pollinated to individuals of the population 500 and vice versa. The plants were dug out from the natural habitat and grown in the area of Ojców National Park. From these crosses 87 seeds were obtained. The measurements of these seeds are demonstrated in the Table 3. The difference between the cross 200 × 500 and 500 × 200 is striking.

Table 3

Seed size and weight in the artificial reciprocal crosses between plants of the natural populations 200 and 500 from the Ojców National Park

Population	No. of seeds	Seed length in mm						Mean weight in mg
		1.7 - 1.5	1.5 - 1.0	1.0 - 0.8	0.8 - 0.6	0.6 - 0.4	0.4 - 0	
200 ♀ × 500	41	3	-	17	3	18	-	0.30
500 ♀ × 200	46	-	-	-	26	12	8	0.06
200	20	-	20	-	-	-	-	0.35
500	24	-	17	3	-	4	-	0.20

There are in the former large seeds and in the latter much smaller ones. The larger seeds, however, weigh more in comparison with the artificial F_1 hybrids. They are rather sound and not so empty, as might be expected in seeds derived directly from the cross of the pure species.

A question arises as to whether the increase in weight of the seeds from 200×500 provided evidence for emphasizing the character of hybrid F_2 or its backcross nature. If so, it might be justifiable to consider the populations 200 and 500 as being of hybrid character.

Further evidence is provided by diads discovered among the tetrads of the pollen cells of certain specimens from the population 200. This indicates disturbances occurring at meiosis.

In order to confirm these conclusions, it will be desirable to make further experiments. It was unfortunate that the chromosome counts in this work were scarce and could not elucidate the question of natural hybrids at the polyploid level.

As Valentine (1963) has shown, there may be triploid hybrids under natural conditions, and these would resemble *P. elatior* more closely than *P. veris*, since they have 2 genomes of the former species to only one of the latter. Further investigation along these lines would certainly be interesting.

RESULTS OF POPULATION STUDIES

The following characteristics were studied. As qualitative characters were taken: colour of corolla and throat, pattern of throat and calyx shape. The colours were indicated by means of the Horticultural Colour Chart (1927). The intermediate traits were sometimes rather difficult to define. As quantitative characters were used: flower size, characterised by the measuring of diameter of the corolla of the largest flower (on the horizontal plane, not the natural one), calyx size — length taken from the teeth to the base and width at the largest mid-point; height — scape with the inflorescence; length of the longest scape; length of the longest pedicel; length of the longest secondary

bract; number of flowers in the largest inflorescence; number of flowers at the time of observation; number of "pin" and "thrum" plants; number of scapes in one rosette; leaf length and width measured in fresh and herbarium material; leaf pubescence in units of binocular (1 mm = 93 units); capsule length and width, number of fully developed capsules in the largest inflorescence; number of seeds in the capsule; seed size and content in mature and half mature material, measured in binocular units (1 mm = 93 units). The seeds were classified as large, medium, small, and also sound (containing embryo and endosperm) to completely empty; weight of the mature seeds; maternal and endosperm layer length, in longitudinal section of the soaked seeds, under the binocular.

Figure 6 represents the distribution of five quantitative outstanding characters in the natural populations of the Ojców National Park, as compared to the populations of the putative parental species. From this figure it is evident that the standard species are clearly distinct as to leaf length. The ranges of values concerning the length of the calyx are to a slight degree overlapping as also the scape length and calyx width. In the number of flowers, there is a considerable overlap. The natural populations of the Ojców National Park show values intermediate between the values of the two species and a relatively high proportion of values which have a pronounced trend to the *P. elatior* type, being the most emphasized in leaf and scape length.

The analysis of variance showed, for the calyx length and width, corolla diameter, leaf length, pedicel length and the number of capsules (Table 4), that all the differences between mean values among both

Table 4

The least significant differences for certain floral, leaf, peduncle and capsule measurements in *P. elatior* and Ojców National Park populations (above) and the same (below) for *P. officinalis* and Ojców National Park population

Population		Corolla diameter		Calyx length		Calyx width		Leaf length	Peduncle length	No. of capsules
		pin	thrum	pin	thrum	pin	thrum			
L.S.P.	<i>P. elatior</i>	0	0	0	0	0	0	0	0	0
	200	1.95	0.35	<u>1.55</u>	1.65	0.35	0.25	<u>6.56</u>	<u>8.32</u>	<u>2.05</u>
	500	<u>9.35</u>	<u>6.75</u>	0.10	1.20	1.00	1.50	<u>4.09</u>	2.28	<u>2.96</u>
	300	<u>4.80</u>	1.30	<u>2.10</u>	2.15	<u>3.25</u>	<u>3.45</u>	<u>5.96</u>	<u>6.53</u>	0.95
	400	<u>7.15</u>	<u>3.80</u>	1.00	1.15	2.40	<u>2.60</u>	2.76	1.91	1.10
	600	<u>5.65</u>	<u>2.55</u>	0.35	0.30	1.25	1.35	0.31	0	<u>3.30</u>
L.S.P.	<i>P. officinalis</i>	<u>8.55</u>	-	<u>5.35</u>	<u>5.50</u>	<u>3.90</u>	<u>4.05</u>	<u>17.90</u>	<u>22.66</u>	6.00
	<i>P. officinalis</i>	0	0	0	0	0	0	0	0	0
	200	<u>6.60</u>	<u>3.90</u>	<u>6.90</u>	<u>7.50</u>	<u>4.25</u>	<u>3.80</u>	<u>11.33</u>	<u>1.43</u>	<u>3.95</u>
	500	0.80	<u>2.55</u>	<u>5.45</u>	<u>6.70</u>	<u>2.90</u>	<u>2.55</u>	<u>13.81</u>	<u>12.78</u>	<u>3.19</u>
	300	<u>3.75</u>	<u>2.90</u>	<u>3.25</u>	<u>3.35</u>	0.65	0.60	<u>11.94</u>	<u>16.13</u>	<u>5.05</u>
	400	1.40	0.40	<u>4.35</u>	<u>4.35</u>	1.50	1.45	<u>15.14</u>	<u>20.75</u>	<u>4.86</u>
L.S.P.	600	2.90	1.65	<u>5.00</u>	<u>5.80</u>	<u>2.65</u>	<u>2.70</u>	<u>18.21</u>	<u>22.66</u>	<u>2.76</u>
	<i>P. elatior</i>	8.55	<u>4.20</u>	<u>5.35</u>	<u>5.50</u>	<u>3.90</u>	<u>4.05</u>	<u>17.90</u>	<u>22.66</u>	<u>6.00</u>

Fig. 6. Histograms of characters in populations of the Ojców National Park in comparison to those of the populations of Tatra and Wielkopolski National Parks

species are significantly greater at the 0.05 level than those between the species and the individuals of the populations of the Ojców National Park. Also, the lowest significant differences between the means of mixed populations and *P. elatior* are smaller than the corresponding differences in relation to *P. officinalis*. The total number of the significant differences for all characters is 45, including 26 non-significant in relation to *P. elatior*, and only 9 to *P. officinalis*. Some attention was given to the heterostyly. Data for long styled (pin) and short styled (thrum) plants treated separately are consistently different for the field populations at the 5 per cent level in the corolla diameter, but are not significant for the calyx size.

The results of pooling the variances for different samples is presented in Table 5. The pooled variances concerned floral, leaf, and scape dimensions, and number of capsules. It may be concluded that there was more homogeneity in the Ojców National Park populations to *P. elatior* than to *P. officinalis*.

Further support for this conclusion comes from the data obtained of the artificial hybridization of certain individuals from the Ojców National Park in comparison with the putative natural F_1 hybrids discovered in this area. Though not many, there did occur, among the natural populations, individuals with larger seeds than the normal (Table 6), including quite empty ones (Table 7). Those individuals belonged to the population 400. They were specimens No. 410 and 411. These plants were as to most characters intermediate. In height like *P. officinalis*, and in corolla quantitative and qualitative traits resembling *P. elatior*. Probably, they were in close approximation to the F_1 hybrids with *P. elatior* as female parent.

Table 5

The pooled variances for certain floral, leaf, peduncle and capsule measurements of *P. elatior* and Ojców National Park populations (above) and the same (below) for *P. officinalis* and Ojców National Park population

Population	Corolla diameter		Calyx length		Calyx width		Leaf length	Peduncle length	No. of capsules
	pin	thrum	pin	thrum	pin	thrum			
<i>P. elatior</i>	12.78	10.99	4.41	5.90	0.37	0.07	7.95	23.04	4.84
200	<u>6.16</u>	<u>11.48</u>	<u>3.38</u>	5.49	<u>0.66</u>	1.32	<u>12.25</u>	21.62	8.24
500	4.26	3.26	1.56	2.36	0.89	1.37	8.12	<u>28.73</u>	12.96
300	4.44	5.74	3.53	2.98	0.72	0.61	<u>16.97</u>	<u>39.69</u>	<u>7.34</u>
400	<u>6.26</u>	8.62	<u>8.70</u>	3.08	1.57	1.02	<u>11.22</u>	<u>39.44</u>	15.29
600	9.74	14.73	22.56	3.62	1.78	4.20	6.76	13.69	6.97
<i>P. officinalis</i>	17.41	10.47	26.83	3.75	0.37	10.96	15.60	28.62	29.27
200	6.16	<u>11.48</u>	3.38	<u>5.49</u>	0.66	1.32	<u>12.25</u>	<u>21.62</u>	8.24
500	4.26	3.26	1.56	<u>2.36</u>	0.89	1.37	<u>8.12</u>	28.73	12.96
300	4.44	5.74	3.53	<u>2.98</u>	0.72	0.61	16.97	<u>39.69</u>	7.34
400	6.26	8.62	8.70	<u>3.08</u>	1.57	1.02	<u>11.22</u>	<u>39.44</u>	15.29
600	9.74	<u>14.75</u>	22.56	3.62	1.78	4.20	6.76	13.69	6.97

Table 6

No. of seeds grouped in four grades of size: largest, large, medium and small (in %)

Population	No. of observed seeds	Largest -over 66 units	Large from 14-65 units	Medium from 7-13	Small from 0-6 units
<i>P. elatior</i>	815	-	81.8	4.0	14.2
<i>P. officinalis</i>	2340	-	83.7	3.6	2.7
Population					
200	1447	-	78.8	8.5	12.7
500	2609	-	62.0	8.7	29.3
300	1658	0.001	66.2	12.2	21.6
400	4084	0.002	47.9	8.2	43.9
600	1967	-	78.7	5.8	15.5

All these results suggest that gene flow between the species has occurred, but since introgression is not easy to detect by conventional statistical methods, as Clifford (1958) stated in his paper on intro-

Fig. 7. Histograms of index values of the populations of the Ojców National Park in comparison to those of the putative parental species

Table 7

Seed size and content of the plants: 410 and 411

Plant No.	Capsule	No. of seeds	Length width (units)	Large			Medium			Small
				sound	with endo-sperm	empty	sound	with endo-sperm	empty	
411	1	1	very large	small embryos 4		1				35
		4	66 × 36							
		8	55 × 24							
		1	46 × 25							
411	2	3	very large	1	1	1			5	42
		2	74 × 54							
		5	66 × 36							
			6 × 12							
411	3	1	very large	1	1	1			2	45
		2	42 × 37							
		1	35 × 32							
		5	28 × 35							
411	4	1	8 × 10	small embryo 1		1				35
		3	25 × 25							
		2	25 × 16							
			6 × 9							
410	1	1	very large	1	1	18				32
		20	57 × 35							
410	2	1	48 × 27		1	9				36
		9	56 × 44							
410	3	9	32 × 20		1	8	6			50
		6	26 × 20							
410	4	9	6 × 8		1	8	3		1	45
		4	26 × 20							
410	5	1	11 × 15	small embryo 2	1	7	10		1	55
		8	61 × 35							
		11	39 × 38							
			8 × 11							
410	6	2	very large	small embryo 2	3	2	1	2		41
		15	25 × 20							
		3	22 × 15							
410	7	2	10 × 12	small embryo 1		1				
		16	24 × 19							
410	8	1	22 × 15	6		11			11	
		17	23 × 17							
		11	13 × 15							

gression between *P. vulgaris* and *P. veris*, therefore, in the present work the method of aggregate hybrid index, used by Anderson in the case of introgression has been additionally applied. The index was computed for each individual. A series of standard scoring values was arbitrarily established, as shown in Table 8. The low values for each

Table 8
Character indices

Character	<i>P. elatior</i>	Score	Intermed.	Score	<i>P. officinalis</i>	Score
Peduncle length (cm)	0 - 14.9	0	15.0 - 29.9	1	30 - 50	2
Pedice! length (mm)	0 - 11	0	12 - 23	1	24 - 50	2
Sec. bract length (mm)	0 - 6	0	7 - 9	1	10 - 15	2
No. of flowers	0 - 5	0	6 - 8	1	9 - 20	2
Calyx length (mm)	0 - 12	0	13 - 16	1	17 - 20	2
Calyx width (mm)	0 - 5	0	6 - 7	1	8 - 15	2
Corolla diameter (mm)	17 - 25	0	13 - 16	1	0 - 12	2
Leaf length (cm)	0 - 14.9	0	15.0 - 22.4	1	22.5 - 40	2
Leaf width (cm)	0 - 3.5	0	3.6 - 5.5	1	5.6 - 9.0	2
Total		0		9		18

character are typical (with the exception of the corolla diameter) for *P. elatior*, and the high ones for *P. officinalis*. Leaf length, and calyx width are more sharply distinct in the standard series than the secondary bract length and the number of flowers. The range of score for nine characters used is from 0—18. The hybrid index values obtained for the standard *P. elatior* and *P. officinalis* as compared with the populations are represented in histograms (Fig. 7). Special attention was paid to the populations of the Ojców National Park, which have more multi-formed individuals, such as population 300, 400 and 600. The standard values are, as was to be expected, discontinuous, with 0—8 as index values for *P. elatior*, and 9—18 for *P. officinalis*. This shows that in spite of overlapping in numerous individual characters, the two standard series of specimens are distinct in the aggregate of the nine characters studied. Specimens of the Ojców National Park which are intermediate or approach *P. elatior*, present a larger number of individuals with relatively low scores than high ones. Some traits of bimodality in the populations from the Ojców National Park constitute further evidence of hybridization. If the combination of characters in the individuals of the Ojców National Park were random, then the number of plants with indices from 0—18 would approach normal distribution. This is not the case, and the distribution of characters in the Ojców National Park plants reveals that the prevailing number of plants are more nearly related to *P. elatior* and have indices ranging from 0—7.

The above results seem to indicate that in the Ojców National Park populations, the segregation of characters does not take place independently, but that sets of characters from putative parental species are inherited "en bloc".

In the Ojców National Park, there are among the plants observed 12 individuals, or approximately 3.4%, which appear to be intermediate between *P. elatior* and *P. officinalis* — namely: 3 in population 300, 8 samples in population 400, and 1 in population 600. By far the majority of plants in the Ojców National Park are predominantly of *P. elatior* characters, but have a few genes from *P. officinalis*. From the above

Fig. 8. Scatter diagram of the populations from the Ojców National Park

data, it seems fairly clear that 12 individuals with hybrid indices intermediate between the scores of the two standard series, are either F_1 hybrids or progeny of such hybrids, which have retained an approximately equal proportion of genes from the two parental species. Their dimensions as to leaf width, which has been the only measurable character from the Valentine's data (1952), approached the measurements of the stock T21e. The mean leaf width of these selected samples from the Ojców National Park ranges from 3.6 to 5.5 cm, as compared with Valentine's samples 4.8—5.9 cm. The remaining, and dominant, 96.6% part of individuals from the Ojców National Park are probably backcross derivatives having genes predominantly of *P. elatior*.

In order to solve the multiple correlation between the characters

a visual method has been used, in which the particular characteristics are presented. In Fig. 8 two characters: corolla diameter and scape length were used as co-ordinates. They distinguish oxlip and cowslip, and are fairly well correlated. The calyx length is also taken into consideration. Corolla, calyx and scape samples from individuals were taken from the 7 chosen habitats of the Tatra, Wielkopolski and Ojców National Parks. Observations and measurements were made on each plant and the data were plotted on the graph: the data of the Ojców National Park specimens with the two polygons representing the limits of the external points corresponding to the species populations. Left polygon involves typical oxlip and typical cowslip in the right one. Typical oxlip has a large corolla and a shorter scape; its calyx extends generally from 7—16 mm. Typical cowslip has a small corolla, a long scape with a longer calyx, mostly from 17—21 mm.

Between these two there is no large clear cut difference. The plants involved in the two species polygons may be attributed to products of backcrossing. They are much more numerous in the direction of *P. elatior* than of *P. officinalis*.

DISCUSSION

Numerous extensive studies on natural populations reveal that introgressive hybridization plays a certain role in the evolution of the flowering plants (Rollins 1954).

Among species between which there are no strong external barriers of isolation, interspecific hybridization occurs frequently. A different situation occurs as between species which have simultaneously external and internal barriers of isolation to a greater or lesser extent. The species of *Primula* from the section *Vernales* show internal and external isolating factors existing together.

Though there are numerous restrictions in the hybridization described above, fertilization does, however, take place and from all crosses seed are produced. It depends on which species is used as female, as is proved by the results of Valentine's intensive experiments, and in part of the experiments of the present work. The F_1 hybrids are difficult to produce, and consequently are rare. On the other hand, F_2 hybrids and the backcrosses are more likely and were more successfully obtained, according to Valentine (1956).

Taking into consideration two populations of two sympatric species, which during evolution diverged probably as a result of geographical isolation, and which became in response to different environment conditions, rather different as regards morphological and physiological characters, came back to the common area, it might in certain cases be possible that gene exchange has taken place.

In the literature, only one example is cited of a natural hybrid of the species studied, discovered by Hill (1907). Valentine (1953), in referring to natural hybrids between *P. elatior* and *P. veris* frequently found in Essex (Melville 1936) emphasized that they need to be more intensively studied. To date, only one work has been published on introgression between species of the section *Vernales* of the genus *Primula* namely between *P. vulgaris* and *P. veris* by Clifford (1958). This author provides no evidence for the introgression between those species, but he concluded that introgression was impossible to detect by traditional biometrical methods only, that is, analysis of variance and the homogeneity test. The present work is the first to investigate the introgression between *P. elatior* and *P. officinalis*, taken place in the populations of the Ojców National Park. It seems that the predominant part of the plants here are backcross hybrids. Few only resemble the artificial F_1 hybrids for example as No. 410, 411, 312 as regards certain characters, and the hybrids with index of 1 also in some respects resemble F_1 hybrids. From the histograms of the hybrid indices and the scatter diagram, it has been found that in the Ojców National Park there are more individuals which approach *P. elatior* than *P. officinalis*, and this strongly suggests that there is a flow of *P. officinalis* genes into *P. elatior*.

SUMMARY

Ninety four individuals of the natural population from the Tatra National Park as *P. elatior* samples, one hundred and one individuals of the Wielkopolski National Park, as *P. officinalis*, and 344 individuals from the populations of the Ojców National Park were studied. Artificial hybridizations experiments were performed on the material transplanted from the natural habitats. The results of the artificial hybridization were poor. The seeds, however, were produced from both *P. elatior* $\times$ *P. officinalis* and the reciprocal crosses. The seeds of the former cross did not germinate and of the reciprocal only 0.05% germinated.

On the basis of the assumption that hybridization may take place in nature, population of the Ojców National Park were examined, where the distribution of both species meets and overlaps.

From the analysis of variances based on six characters, it was found that there were significant differences at the 5% level between individuals of the Ojców National Park and *P. officinalis*, and the homogeneity to *P. elatior* was greater than to the other species.

Some plants of the natural populations from the Ojców National Park reveal subnormal and empty seed, similarly to the plants obtained from the artificial hybridization.

A hybrid index based on 7 characters from the autumn observations and 9 characters from the flowering time, made it possible to separate among the populations presented, 3.4% putative F_1 hybrids, and the majority as segregants of the later progenies and backcrosses, of which a great number showed values similar to *P. elatior*.

Additional data revealing the same situation are shown in a scatter diagram. It is thus concluded that the introgression that is natural gene-flow from *P. officinalis* into *P. elatior* does take place in the Ojców National Park.

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Introgresja pomiędzy Primula elatior (L.) Hill. i *Primula officinalis* L.

Streszczenie

Obrano jedną populację w Tatrzańskim Parku Narodowym, jako stanowisku naturalnym *P. elatior* i drugą w Wielkopolskim Parku Narodowym, jako stanowisku *P. officinalis* oraz 344 osobniki w 5 populacjach na terenie Ojcowskiego Parku Narodowego. Wykonano krzyżówki na materiale przesadzonym ze stanowisk naturalnych. Wyniki krzyżowania okazały się nikłe, ale otrzymano nasiona zarówno z krzyżówki *P. elatior* × *P. officinalis*, jak i odwrotnej. Nasiona pierwszej krzyżówki jednakże nie skielkowały, a odwrotnej zaledwie w 0,05%.

Na tej podstawie przypuszczono, że krzyżowanie w naturze, może też występować. Zbadano więc populacje z Ojcowskiego Parku Narodowego, gdzie zasięgi obu gatunków się stykają lub zachodzą na siebie.

Z analiz wiariancyjnych 6 cech stwierdzono istotne różnice na poziomie 5% pomiędzy osobnikami z Ojcowskiego Parku Narodowego i *P. officinalis*, większe niż z *P. elatior*, a testy jednorodności potwierdzały te wnioski.

Przy pomocy metody wskaźników mieszańcowości, opartej na 7 cechach z obserwacji jesiennych i na 9 cechach z okresu kwitnienia, okazało się możliwe wyróżnienie spośród osobników populacji z Ojcowskiego Parku Narodowego 3,4% przypuszczalnych mieszańców F_1 i większości segregantów dalszych pokoleń lub mieszańców wstecznych, z których najwięcej przedstawiało cechy zbliżone do *P. elatior*.

Na podstawie zgodności wyników, opracowanych kilku różnymi metodami, stwierdzono występowanie introgresji pomiędzy osobnikami z Ojcowskiego Parku Narodowego i przepływu genów od *P. officinalis* do *P. elatior*.